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TOPIC 15 – Electrophysiology, arrythmias and pacing – C

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Impaired clathrin-mediated endocytosis of Kv1.5 channels in dilated atria?

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Mechanisms responsible for atrial fibrillation (AF) are complex. At tissue and cellular levels, Ca^{2+} currents are down-regulated whereas repolarizing K^+ currents are maintained, leading to action potential shortening. At the protein level however, Kv1.5 channels, the molecular expression of I_{Kur}, the main repolarizing current in human atria, is significantly decreased. We used a rat model of atrial remodeling associated with heart failure reproducing AF substrate to investigate the mechanisms responsible for the discrepancy observed between maintained I_{Kur} and decreased Kv1.5 protein. We thus investigated: 1) the endocytosis pathway of Kv1.5 channels in the atria; and 2) the internalization activity in the dilated atria. 3-D microscopy revealed that Kv1.5 channels are associated to clathrin vesicles. Electron microscopy (EM) showed that vesicles are found both at the lateral membrane and at the intercalated disc. Blockade of the clathrin pathway using hypertonic media or SiRNA induced an increase in I_{Kur}, an accumulation of Kv1.5 channels at the sarcolemma (biotinylation) and an increased fluorescence recovery (FRAP), supporting that Kv1.5 channels are internalized by the clathrin pathway. In dilated atria, Kv1.5 protein level is decreased by 25% whereas I_{kur} density is unchanged, suggesting that Kv1.5 channels accumulate at the sarcolemma. Therefore, we looked at clathrin activity in dilated atria. We found a 35% reduction of clathrin levels. Moreover, Kv1.5 channels were less associated with clathrin vesicles in dilated myocytes. However, EM showed no significant difference in the internalization activity between sham and dilated atria. Therefore, the reduced clathrin protein synthesis observed in dilated atria is not likely responsible for the accumulation of Kv1.5 channels in the sarcolemma. Other mechanisms such as increased recycling and/or membrane stabilization must be investigated to understand how I_{Kur} is maintained in dilated atria and during chronic AF.

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Shear stress triggered recruitment of potassium channels to the plasma membrane of atrial myocytes is dependent upon integrin activation

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During the cardiac cycle atrial myocytes are continuously exposed to shear stress (SSt), occuring as laminar sheets of cells move relative to each other. Physiological levels of SSt can modulate activity and cellular localisation of ion channels. However, in atrial myocytes the effects of SSt are poorly known. Here, we investigate the effect of SSt on the atrial repolarisation potassium (K+) current. Whole cell outward currents were recorded from rat atrial myocytes while they were subjected to SSt of 0.5 dyn/cm^2. Increasing SSt to 4.5 dyn/cm^2 induced a progressive increase in current from 3.8 pA/pF±0.5 to 43.2 pA/pF±8.9. The effect was reversible and was inhibited by the K+ channel blocker 4-AP at a concentration which specifically blocks Kv1.5, the channel underling IKur. Myocytes exposed to SSt showed a shortening in action potential duration consistent with an increase in IKur. We have previously shown that Kv1.5 can be recruited to the plasma membrane (PM) from a subcellular pool, *via* a Rab11 dependent process. The increase in current induced by SSt was attributed to increased trafficking of channels from these pools, as it was inhibited by N-ethylmaleimide, which inhibits fusion of vesicles to the PM. In addition, the SSt response was attenuated by both colchichine, which destroys the microtubule network, and cytochalasin D which inhibits actin polymerisation, indicating a role for the cytoskeleton in mediating the effect. The integrin inhibitor echistatin and the focal adhesion kinase inhibitor (FAK) FAKi14 both prevented the SSt effect, suggesting that this integrin signalling pathway is the mechanotransduction mechanism. Interestingly, SSt alone was able to activate FAK as shown by an increase in its phosphorylation. In conclusion, SSt induces the recruitment of Kv1.5 channels to the PM *via* an integrin/FAK dependent process. Thus, pools of Kv1.5 may comprise an inducible reservoir which can repolarise the atrium under conditions of mechanical stress.

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***ABCC9* and early repolarization syndrome**

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Until recently, early repolarization (ER) has been considered as a benign electrocardiographic pattern. Recent studies conclude that individuals with ER in infero-lateral leads are at higher risk of developing ventricular arrhythmia and sudden cardiac death. Therefore, the potential malignant nature of ER syndrome (ERS) cannot be neglected anymore. ERS phenotype has been correlated to mutations of different genes including *KCNJ8*, encoding the predominant cardiac K_{ATP} channel pore-forming subunit, Kir6.1.

The cardiac K_{ATP} channel is a heteromeric complex of Kir6.1 or Kir6.2 (encoded by *KCNJ11*) subunits and regulatory subunits SUR2A (*ABCC9*).

Little is known about the extent to which K_{ATP} mutations contribute to ERS associated with sudden cardiac death. The purpose of this study was to explore *ABCC9* as a novel susceptibility gene for ERS.

Direct DNA sequencing of *ABCC9* was performed among 94 probands diagnosed with ERS or idiopathic ventricular fibrillation. Three rare mutations were identified in *ABCC9* (leading to p.A665T, p.V1137I, and pV1319I) and functionally characterized. Mutant SUR2A cDNA-containing plasmids were co-transfected with Kir6.x subunits cDNA-containing plasmids in COS-7 cells. Wild type (WT) and mutants K^+ currents were recorded using the whole-cell configuration of the patch clamp technique (20°C).

We observed no effect of SUR2A V1137I or V1319I variants on the K^+ current when compared to WT SUR2A, whatever the co-expressed Kir6.x subunit. However, K_{ATP} current of Kir6.1-SUR2A-A665T in the presence of 100 µM pinacidil was significantly increased, compared to Kir6.1-SUR2A WT current (22.7±3.3 pA/pF, n=29 vs 63.8±17.3 pA/pF, n=17, for WT and A665T, respectively; $p<0.05$, Mann-Whitney rank sum test), but when expressed with Kir6.2, no difference could be detected.

In this study, we identified *ABCC9* as a novel ERS susceptibility gene and a mutation leading to a marked gain-of-function of the cardiac K_{ATP} Kir 6.1-SUR2A channel complex.

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Numerical simulation of the electrical activity of the heart from ion-channel to body surface ECGs: Modelling and applications

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Meaningful computer based simulations of the ECG, linking models of the electrical activity of the heart to ECG signals, are a necessary step towards the development of personalized cardiac models from clinical ECG data. An ECG simulator is, in addition, a valuable tool for building a virtual data base of pathological conditions, to test and train medical devices but also to improve the knowledge on the clinical significance of some ECG signals.

We developed a 3D computational model of the electrical activity of the heart based on the state-of-the-art mathematical models. A 3D anatomically-based model of the whole human body is presented with biophysically-detailed representation of human membrane kinetics, realistic cardiac geometry, fibre orientation and cell

heterogeneity in electrophysiological properties of cardiac ventricles. This mathematical model is used in different application. We present two different applications using our ECG simulator.

Electro-pharmacology: We use the 3D model to simulate the effect of specific drug concentrations of fast sodium, hERG current and L-type calcium blockers on different levels. In particular we show the drug effect on the action potential at the cell level, on activation and repolarization maps at the heart level, and on the Qt interval at the body surface level. The simulation of drugs effect is also shown on different other biomarkers.

Electrocardiography: The main goal is to construct the electrical potential on the heart surface from ECG-like measurements on the body surface. The potential data is later exploited to build activation maps on the heart surface. This problem is difficult to solve and is known to be mathematically ill posed. We use the ECG simulator in order to simulate different scenarios of healthy and pathological cases. The generated synthetic data is later used in order to test different mathematical approaches introduced to solve the inverse problem.

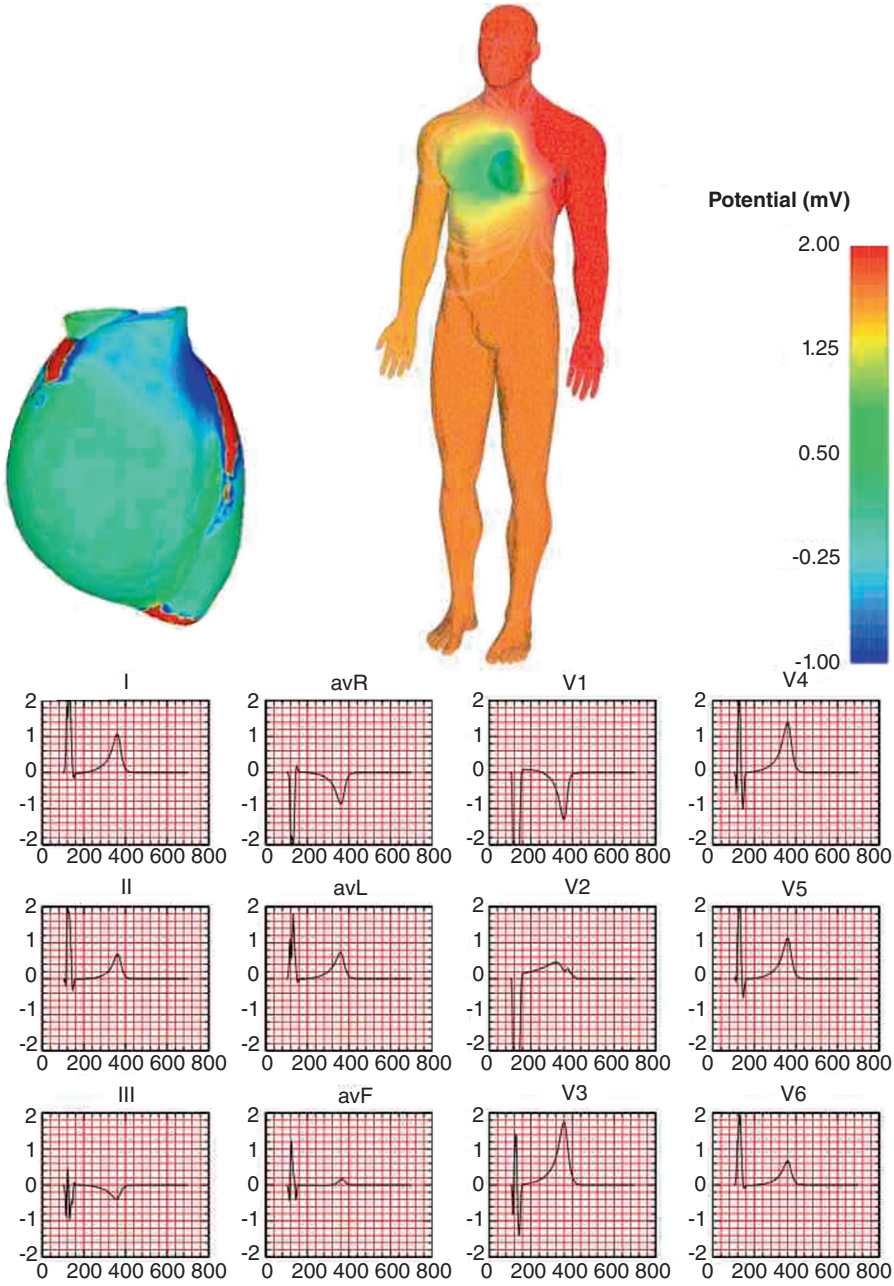

Figure – Abstract 143 – Body surface potential snapshot and ECG plot.

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Aggravation of myofibroblast arrhythmogeneicity by mechanical stress

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Due to their low membrane polarization, cardiac myofibroblasts (MFBs) induce arrhythmogenic slow conduction and ectopic activity by causing partial depolarization of electrotonically coupled cardiomyocytes (CMCs) in-vitro. We hypothesize that mechanical stress may aggravate this condition by activation of stretch activated channels (SACs) in MFBs that accentuate MFB depolarization. Single and multicellular preparations were obtained from neonatal rat ventricular cells. Acutely blocking SACs with streptomycin (SM) (50 µMol/L) had no effect on Vm of single CMCs (control: -75.1±2.62mV; SM: -75.3±2.59mV, n=8), but caused MFBs to hyperpolarize from -33.7±11.2 mV to -37.7±11.4 mV (n=8) suggesting the presence of active SACs in MFBs under control conditions. Accordingly, SM had no effects on impulse conduction (θ) in CMC cell strands (control: 336.3±24.4 mm/sec; SM: 329.5±21.4 mm/sec; n=58; n.s.) but increased θ from 173.8±68.2 mm/sec to 224.9±63.3 mm/sec (n=54; p<0.0001) in strands of CMCs coated with MFBs. Modulation of θ by MFB-SACs was observed by subjecting cell strands cultured on silicone membrane to acute length changes. In CMC-only strands, θ was positively correlated with strand length (10% relaxation: 325.7.6±26.4 mm/s; control: 335.8±27.8 mm/s; 10% stretch: 348.8±39.9 mm/s; n=25) which can be explained by changes in cell geometry. In contrast, θ of hybrid CMC-MFB strands decreased with increasing strand length (10% relaxation: 291.4±48.7 mm/s; control: 276.7±44.2 mm/s; 10% stretch: 255.0±46.6 mm/s, n=20) suggesting that stretch caused activation of SACs in MFBs that ultimately led to partial depolarization of coupled CMCs. The results demonstrate that acute stretch differentially affects θ depending on the cellular composition of cardiac tissue. Slowing of conduction is observed only in presence of MFBs suggesting that arrhythmogenic consequences of mechanical stress may be aggravated by MFBs in fibrotically remodelled myocardia.

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MRI assessment of mechanical dyssynchrony in patients with heart failure and narrow QRS

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Introduction: Cardiac resynchronization therapy (CRT) is not indicated in patients (pts) with narrow QRS (≤120 ms), although some may have evidence of mechanical dyssynchrony (MD) and may benefit from CRT. We used an MRI-derived systolic dyssynchrony index (SDI) for volume change to assess MD in heart failure patients with a narrow QRS, and delayed enhancement imaging to assess for myocardial scar so as to evaluate the prevalence of MD in such patients and the effect of scar extent on MD.

Methods: 50 pts with LVEF<35% and narrow QRS underwent MRI. Contour tracking software (TomTec; Munich) was used to generate time-volume curves based on a 16-segment model of the LV from cine SSFP images, using semi-automatic border detection. The software calculated the SDI (standard deviation of time to peak volume change for the 16 LV segments expressed as a percentage of the RR interval; greater SDI reflects greater MD). SDI ≥10.0% was used as a cut-off based on previous work with echo-derived SDI indices. Myocardial scar extent was quantified using delayed-enhancement MRI. The association between MD and scar in pts with HF and narrow QRS was explored.

Results: Mean QRS duration was 107±10 ms. Mean LVEF was 27±8%. Mean SDI was 8.3±4.1% (range from 3.1 to 22.1%). 24% of these pts had MD with a mean SDI of 14.2±3.6% (range from 10.2 to 22.1%) with a mean QRS duration of 110±12 and a mean LVEF 28±7%. Pts without MD had a mean SDI of 6.4±1.8% (range from 3.1 to 9.0%) with a mean QRS duration of 106±10 ms and a mean LVEF of 29±6%. 49% of pts with narrow QRS had ischemic scar with a mean LV scar extent of 17.9±8.9% (Qmass software). The mean SDI didn't differ significantly in pts with ischemic scar and in pts without scar (7.9±3.2 and 7.0±2.8 respectively, p=0.958). In pts with ischemic scar, SDI was not correlated to scar extent (R=0.036; p=0.879).

Conclusion: Pts with HF and narrow QRS have a wide spread of SDI derived from cardiac MRI and 24% of these pts have MD. The presence of scar doesn't predict MD. The role of CRT is these patients should be prospectively investigated.